Putting Tumors in Context

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The interactions between cancer cells and their micro- and macroenvironment create a context that promotes tumour growth and protects it from immune attack. The functional association of cancer cells with their surrounding tissues forms a new 'organ' that changes as malignancy progresses. Investigation of this process might provide new insights into the mechanisms of tumorigenesis and could also lead to new therapeutic targets.

Under normal conditions, ORGANS are made up of TISSUES that exchange information with other cell types via cell–cell contact, cytokines and the EXTRACELLULAR MATRIX (ECM). The ECM, which is produced by collaboration between STROMAL fibroblasts and EPITHELIAL cells, provides structural scaffolding for cells, as well as contextual information. The endothelial vasculature provides nutrients and oxygen, and cells of the immune system combat pathogens and remove apoptotic cells. Epithelial cells associate into intact, polarized sheets. These tissues communicate through a complex network of interactions: physically, through direct contact or through the intervening ECM, and biochemically, through both soluble and insoluble signalling molecules. In combination, these interactions provide the information that is necessary to maintain cellular differentiation and to create complex tissue structures.

Occasionally, the intercellular signals that define the normal context become disrupted. Alterations in epithelial tissues can lead to movement of epithelial sheets and proliferation — for example, after activation of mesenchymal fibroblasts due to wounding.Normally, these conditions are temporary and reversible, but when inflammation is sustained, an escalating feedback loop ensues.Under persistent inflammatory conditions, continual upregulation of enzymes such as matrix metalloproteinases (MMPs) by stromal fibroblasts can disrupt the ECM, and invading immune cells can overproduce factors that promote abnormal proliferation. As this process progresses, the normal organization of the organ is replaced by a functional disorder (FIG. 1). If there are pre-existing epithelial cells within this changing context that possess tumorigenic potential, they can start to proliferate. Alternatively, the abnormal interactions might lead to genomic instability within the epithelial cells and the acquisition of tumorigenic potential. The proliferating cancer cells can then interact with their microenvironment and enhance the abnormal interactions. At this point, the tumour has become its own organ,with a distinct context that now defines all its cellular responses.Here,we will examine how the mechanisms that contribute to the normal context also act to suppress developing tumours, how disruption of this context initiates and supports the process of tumorigenicity, and how some cells with a tumorigenic genotype can become phenotypically normal if the context is appropriately manipulated.

An innate anticancer mechanism

An important feature of the normal stromal context is the generation and maintenance of epithelial-cell polarity. Epithelial cells receive a variety of orientational cues from the environment that help them establish cellular apical and basal surfaces and to maintain the differentiated state. Loss of polarity has been shown to lead to increased cell proliferation and tumorigenesis (BOX 1).The basal surface of epithelial cells associates with the BASEMENT MEMBRANE, a specialized form of ECM that provides both structural support and polarization signals to epithelia. The basement membrane is a dynamic structure. Changes in its composition lead to changes in cell shape and behaviour[1], altered binding affinity or cellular distribution of

cell-surface receptors[2], and cellular responses to soluble molecules[3].Depending on the composition and physical characteristics of the basement membrane, different soluble factors can have completely different cellular effects, such as inducing cell proliferation, growth arrest, differentiation or apoptosis[4].

Epithelial cells maintain physical contact with their neighbours through a combination of ADHERENS JUNCTIONS, GAP JUNCTIONS, TIGHT JUNCTIONS and DESMOSOMES (FIG. 2).Of these, adherens junctions have been a particular focus of studies into the signals that generate epithelial-cell polarity, but more recent investigations have revealed mechanisms by which gap junctions, tight junctions and desmosomes also contribute to the formation of polarized epithelial tissues[5–8].

Adherens junctions are contacts between adjacent epithelial cells and are anchored to the cytoskeleton. Cadherins (such as E-CADHERIN) traverse the membrane, associating with cadherins on adjacent cells in a calcium-dependent manner. On the cytoplasmic face, β-catenin connects to the cadherin tail and associates with α-catenin,which in turn binds to actin. Loss or alteration of these components leads to premalignant phenotypes and even tumorigenesis[9–11].Of these components, E-cadherin has been a particular target of study, as this molecule is lost in many types of tumour[9], and its restoration can suppress cellular transformation. Decreased E-cadherin function is a component of EPITHELIAL–MESENCHYMAL TRANSITION, invasive tumour growth and metastasis[11–13]. Loss of E-cadherin can be accompanied by increased expression of alternate cadherin isoforms that promote inappropriate survival signals and enhance the malignant phenotype[14].However, as all cellular responses are tissue- and context-dependent, there can be no universal generalizations, as shown by the fact that E-cadherin gain of function is an early step in ovarian carcinoma[15].

Gap junctions are channel-forming complexes that allow passive diffusion of small signalling molecules between neighbouring cells[5].The particular composition of CONNEXIN subunits within a gap junction determines the type of molecule that can be transported[16]. Much remains to be learned about how the specific combination of connexins facilitates tissue interactions, but it is clear, again, that generalizations should be avoided, as the expression patterns (and probably the function) of connexins are tissue dependent and change during tumour progression[17,18]. For example, some breast cancer cells upregulate connexin 32 (Cx32)[19], but loss of Cx32 contributes to hepatocellular carcinoma[20,21]; Cx43 inhibits tumorigenicity of lung, cervical and bladder carcinoma cells[22–24], but has no effect on squamous cell carcinomas[25]; and other connexins can facilitate cell adhesion during metastasis[26].

Changing interactions between adjacent tissues might also affect tumour development (FIG. 3). For example, in the normal human mammary gland (FIG. 1), the ductal–lobular system is composed of an inner layer of luminal epithelial cells, which line the duct and produce milk during lactation, and an outer layer ofmyoepithelial cells, which express a number of proteases during tissue remodelling to pave the way for emerging ductules. This double-layered structure is separated from the INTERSTITIAL MATRIX by an intact basement membrane[27]. Breast cancer arises mainly in the luminal epithelial compartment, but myoepithelial cells also express molecules that have been shown to suppress transformation of luminal epithelial cells *in vivo*[27] (TABLE 1). These proteins have been named ‘class II tumour suppressors’[28] and production

of these proteins allows myoepithelial cells to act as tumour suppressors in the breast[27,29].

In combination, these mechanisms create a dynamic equilibrium that helps cells to maintain a normal, differentiated phenotype. This equilibrium might attenuate the consequences of genetic mutations, as consideration of the frequency of spontaneous mutations indicates that many epithelial cells should possess oncogene-activating mutations, yet cells continue to function normally[30,31].Analyses of normal epithelial tissue adjacent to tumours have shown that similar patterns of mutations can be found in both, indicating that malignant cells can exist within normal tissues but be restrained by normal contextual cues[32–34].

Activated stroma as a carcinogen

Whereas normal stroma can delay or prevent tumorigenesis, abnormal stromal components can promote tumour growth (FIG 4).Acquired or inherited mutations that alter stromal-cell function can release the suppression placed on context-inhibited malignant cells. Literature that spans more than a century has shown that inflammation associated with tissue wounding can produce tumours (REFS 35–38 and references therein) (BOX 2). .Barcellos-Hoff and colleagues[39] have shown that irradiation of the mammary-gland stromal component promotes the tumorigenic potential of non-irradiated epithelial cells. These investigators had previously shown that even low levels of irradiation lead to remodelling of the ECM in breast tissue and activation of latent transforming growth factor-β (TGF-β), which affects tissue and organ function[40].Moinfar *et al.*[41] examined genetic alterations in tumour-associated stroma from several independent cases of mammary carcinoma, and found chromosomal rearrangements that were not present in the malignant carcinoma cells. These results indicate that characteristic mutations that affect stromal cells might have contributed to the formation of the epithelial tumours.Moreover, studies of a subset of inherited cancer-susceptibility syndromes[42,43] also indicate that alterations in stromal cells can contribute to tumorigenesis. So, aberrations in stroma can both precede and stimulate the development of epithelial cancers[44,45].

Matrix metalloproteinases

MMPs can degrade ECM and are involved in promoting the inflammatory response, normal tissue remodelling, wound healing and angiogenesis[46]. These enzymes, however, also have an important function in malignancy (BOX 2). The sustained presence of these proteinases in the tumour environment, produced both by the activated cells and by the cancer cells, leads to destruction of normal ECM. Degradation of ECM stimulates both proliferative and apoptotic mechanisms, which can lead to the selection of apoptosis-resistant carcinoma cells and enhanced invasive potential[47,48]. In the tumour context, direct association of MMPs with specific ECM receptors provides spatial control of MMP activity and directional signals to the invading tumour cells[49].

Stromelysin-1 (SL-1, also known as MMP-3), is an MMP that is involved in both mammary-gland development and breast cancer[50,51]. Cellular context determines the response of mammary epithelial cells to SL-1 treatment: when grown in basement-membrane gels, mammary epithelial cells undergo growth arrest and become functionally differentiated; subsequent treatment of these cells with SL-1 causes apoptosis[52]. However, when cultured on two-dimensional matrices

and allowed to continuously proliferate, mammary epithelial cells react to treatment with SL-1 by undergoing an epithelial–mesenchymal transition and becoming tumorigenic[50].

In transgenic mice that express SL-1 in mammary luminal epithelial cells, the mammary glands show morphogenesis defects and contain pre-neoplastic lesions[44,53,54] that eventually lead to full malignancies[50,54]. Here, the causative mechanism seems to be that SL-1 — expressed ectopically at low levels in the epithelial cells — is subsequently produced at much higher levels by the stromal fibroblasts[44], showing that a moderate disruption contributes to a self-sustaining tumorigenic state. Similar reciprocal feedback mechanisms have been observed in transgenic mice with altered expression of MMP-7 (REF. 55), MMP-11 (REF. 56) and MT1-MMP57.

Immune function in the tumour context

Immune surveillance is the mechanism by which the immune system targets and destroys developing malignancies. Investigations of transgenic mice with deficient responses to interferon-γ (IFN-γ), a cytokine that has been shown to be required for migration of T cells to tumour sites[58,59], have led to increased interest in the mechanisms by which immune cells target tumours[60,61]. Although T cells seem to be the main effectors of immune surveillance[62], the innate immune system (which includes natural killer cells, macrophages, monocytes and mast cells) is also involved[63–65].

Malignant cells evade immunosuppression by downregulating intrinsic immunogenicity[66,67]. The tumour vasculature contributes to this process by preventing extravasation of the antitumour T cells, while continuing to allow the passage of innate immune cells[68]. Studies by Gloria Heppner and colleagues (REF. 69, and references therein) showed that natural killer cells actually provided positive signals for progression of preneoplastic mammary lesions. This initially controversial concept has received support from recent investigations of carcinomas of the skin[70,71], pancreas[72] and mammary gland[73], showing that innate immune cells promote tumorigenesis by producing MMPs, inducing the stroma to produce MMPs and by activating latent MMPs that are present in the ECM[74–76]. The resultant increase in proteolytic activity potentiates tumour progression by further degrading ECM, activating tumour-associated fibroblasts and enhancing angiogenesis[70,72].

Macrophage migration inhibitory factor (MIF) is another immunomodulator that is associated with tumour progression. This cytokine has been shown to be overexpressed by tumour cells[77], contributing to neoangiogenesis and to epithelial cell proliferation[77], as well as suppressing immune surveillance[78]. MIF might also contribute to the genomic instability within tumours, as MIF suppresses p53 function[79], potentially leading to the attenuation of normal apoptosis and growth-arrest mechanisms and allowing for the accumulation of additional oncogenic mutations[80]. This might be one of the mechanisms by which persistent inflammation can increase the risk of cancer[81].

Tumour-cell plasticity

One manifestation of the distinct tumour context is that cells from a given malignant tissue are not limited to that tissue's normal panoply of physiological processes. The classic work of Beatrice Mintz (discussed below) is a prime example of this, but a more recent example can be found in 'vasculogenic mimicry', a process in which aggressive tumours can augment normal angiogenesis by forming hollow channels that connect to the existing vascular system.These vessels are believed to transport blood into the depths of the tumour[82,83].This concept has now been well-characterized[84–87] and could represent a general component of tumour development[88].To produce more selective antiangiogenic therapies, it might be necessary to combine detailed examinations of vasculogenic mimicry with existing models of tumour angiogenesis.

Haematological tumours

More than 80% of human cancers are derived from the epithelium, but the role of context in the development and maintenance of cancer also seems to apply to tumours of haematological origin. Although most immune cells spend much of their lifespan in the circulatory system, key aspects of immune-cell development involve cell–cell and cell–ECM interactions within the stroma of the bone marrow, the thymus and the lymph nodes[89,90]. In haematopoietic cells, as in epithelial cells, these interactions control cell shape, adhesion and migration[91].

Accordingly, defects in the function of bone-marrow stromal cells can cause a predisposition to cancer, such as in cases of Shwachman–Diamond syndrome, an inherited preleukaemic disorder that is caused by a faulty bone-marrow microenvironment[92]. As with tumours that are derived from the epithelium, haematological tumour cells interact with their stromal microenvironment through cell-surface receptors[93–95]. These interactions lead to increased production of MMPs[96,97], altered expression of ECM receptors[98,99] and increased angiogenesis[100,101]. The interactions between haematological tumour cells and the tumour stroma are, therefore, a significant component of tumour growth and resistance to anticancer therapeutics[102–106].

Restoring the normal context

Although an abnormal context can contribute to tumorigenesis and tumour progression, there is no compelling evidence that this process, once initiated, is irreversible. The possibility that reintroduction of the normal context could suppress the transformed phenotype was first suggested by the work of Mintz and Illmensee, who showed that TERATOCARCINOMA CELLS, even after prolonged passage, were still capable of differentiating and generating normal mice[107]. This seminal observation indicated that maintenance of a normal context could lead to inhibition or even reversion of tumours *in situ*. In another example, Rous sarcoma virus — one of the most potent oncogenic viruses — is not tumorigenic in the early embryo[108], but when the embryonic cells that host the virus cells are put in culture, they become transformed[109]. In co-culture assays, normal stromal cells inhibit the progression to epithelial malignancy[110]. Norbert Fusenig and colleagues have developed an assay to model the natural tissue context of the stratified skin epithelium[111]. Using this system, they were able to suppress early stages of neoplastic progression of malignant keratinocytes by introducing an excess of normal keratinocytes[112].

An assay involving a three-dimensional (3D) basement membrane[113] has been used to investigate the response of a series of human breast-tumour cell lines at different stages of progression, cultured within a physiological context[114]. Although the nonmalignant cells are similar in appearance to the malignant cells when cultured on plastic substrata, the phenotypic differences are striking when the cells are cultured in a reconstituted basement membrane (rBM)[115].Under these conditions, the non-malignant cells undergo growth arrest and form a polarized, alveolar structure, whereas the malignant cells proliferate and form amorphous structures. Analysis of ECM and growth-factor receptors in the non-malignant and malignant cell types indicates that the malignant cells overexpress INTEGRINS and epidermal growth factor receptor (EGFR). Addition of anti-β1-integrin antibodies to the malignant cells, when cultured in 3D rBM, downregulated EGFR expression, restored cellular organization, and decreased overall tumorigenicity[115]. This observation led to the discovery of a bidirectional cross-modulation of integrin and EGFR signalling that exists only when cells are cultured in 3D[116]. Furthermore, the tumorigenic phenotype of the malignant cells was reversed by treatment with EGFR-inhibitory antibodies, mitogen-activated protein kinase (MAPK) pathway inhibitors, or phosphatidylinositol 3-kinase (PI3K) pathway inhibitors[116,117]. Inhibiting several different signaling pathways restores even an aggressive breast-cancer cell line to a normal phenotype[117].

Therefore, assays in which tumour cells are cultured in physiological conditions can be used to identify combinations of signalling inhibitors with the potential to reverse the progression of a broad range of tumours. The success of agents that are designed to inhibit other signal transduction pathways, such as herceptin (which blocks signalling by the EGFR ERBB2 (HER2) in breast cancer cells) and STI-571 (which inhibits BCR–ABL kinase activity in chronic myelogenous leukaemia cells) indicates that this might be a valid approach[118,119]. It is clear, however, that relapses occur and many patients do not respond. These agents were designed to target one particular oncoprotein, so it might be necessary — in cases of more complex cancers — to target both the tumour and its context, using combinations of drugs.

Targeting the tumour organ

The efficacy of targeting the tumour organ can be found in recent strategies for treating hepatocellular carcinoma. This cancer type is accompanied by a fibrotic stromal reaction in which HEPATIC STELLATE CELLS show increased proliferation, fibrogenesis and matrix degradation, as well as reduced retinoid production and cytokine release[120] — physiological responses often found in tumour tissues. Recent clinical studies indicate that chemotherapy for hepatocarcinoma could be more effective if therapies to target the underlying liver fibrosis were also employed[120,121].As fibrotic breast disease is also associated with a predisposition to breast cancer[122], and environmentally induced fibrotic disorders of the lung can increase incidence of lung cancer[123], targeting the tumour environment might also increase the treatment effectiveness for these types of cancer.

Antagonism of the developing tumour context also offers potential for cancer prevention therapies[124,125]. In the best-characterized example of this approach so far, chronic suppression of inflammation through use of non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to lower the incidence of colon and breast cancer[125,126]. This antitumour activity seems to occur through inhibition of cyclooxygenase-2 (COX2)[127–129], an enzyme that is involved in the

synthesis of pro-inflammatory prostaglandins (see the review by Rajnish Gupta and Raymond DuBois on pp. 11–21 in this issue). The demonstration of the role of COX2 in tumorigenesis serves as a remarkable example of how the several tissue types can collaborate to promote tumour progression, as fibroblasts, immune system cells and cells involved in neoangiogenesis are all part of this pathway[130].

The requirement of tumours for a vascular supply has also produced a diverse group of angiogenesis inhibitors that are currently undergoing evaluation in the clinic[131]. Similarly, the role of MMPs in tumorigenesis, tumour invasion and metastasis has prompted clinical testing of MMP inhibitors.Although the results with patients suffering from advanced stages of cancer have shown no clinical efficacy, recent data indicate that MMP inhibitors could be more successful when used in early-stage cancer or in conjunction with traditional treatment methods[132,133].

So, agents that target the tumour microenvironment represent an important new direction for cancer therapy. Just as the normal context creates a dynamic equilibrium to maintain normal tissue function, so the tumour context contains many overlapping mechanisms to maintain its functional disorder and to evade anticancer therapies. Therefore, it is likely that combinations of the next-generation therapeutic agents, targeting specific molecular targets,will be required not only to inhibit and destroy the tumour cells, but also to normalize the tumour microenvironment. Gaining a better understanding of the complexities of the tumour context will improve our prospects for developing effective cancer treatments. Dormant metastases are not the only sheep in wolves' clothing — the altered microenvironment of the tumour is itself a powerful and insidious carcinogen that needs to be targeted.

Keywords

ORGAN
An anatomically discrete collection of tissues, integrated to perform specific functions.

TISSUE
A relatively homogenous structure, composed of an organized collection of cells of similar morphology and function.

EXTRACELLULAR MATRIX (ECM).
A complex, threedimensional network of very large macromolecules that provides contextual information and an architectural scaffold for cellular adhesion and migration.

STROMA
Organ compartment serving as the connective tissue framework; includes fibroblasts, immune defence cells and fat cells.

EPITHELIUM
A diverse group of tissues that covers or lines nearly all body surfaces, cavities and tubes, functioning as interfaces between different biological compartments. Epithelial layers provide physical protection and containment, and also mediate organ-specific transport properties.

BASEMENT MEMBRANE
A specialized form of ECM that consists of laminins, collagen IV, nidogen (entactin), proteoglycans and a number of other glycoproteins that separates epithelia from underlying supporting tissues. Different organs have different compositions of basement membrane.

ADHERENS JUNCTION
A physical junction that links apicolaterally localized belts of actin in adjacent epithelial cells.

GAP JUNCTION
An aqueous channel that interconnects the cytoplasms of adjacent cells and allows direct exchange of small cytoplasmic components. It is created by the association of two hemichannels, each a hexamer of connexin subunits.

TIGHT JUNCTION
A component of cell–cell adhesion in epithelial and endothelial cell sheets. Acts as a mediator of the diffusion of solutes through the intercellular space. Also acts as a boundary between the apical and basal plasma-membrane domains.

DESMOSOME
An adhesive junction that anchors intermediate filaments between adjoining cells.

E-CADHERIN
The main adhesion receptor in adherens junctions. Mediates Ca2+-dependent interactions between adjacent epithelial cells and regulates cell proliferation. It also sequesters the transcriptional co-activator β-catenin, a protein that can stimulate cell-cycle entry. The loss of E-cadherin from the cell surface might trigger epithelial–mesenchymal transition.

EPITHELIAL–MESENCHYMAL TRANSITION
Conversion from an epithelial to a mesenchymal phenotype, which is a normal component of embryonic development. In carcinomas, this transformation results in altered cell morphology, the expression of mesenchymal proteins and increased invasiveness.

CONNEXIN
Functions as a subunit of the gap junction hemichannel. Several members of the connexin family have been identified.

INTERSTITIAL MATRIX
The extracellular matrix (ECM) contained within the stroma.

INTERMEDIATE FILAMENT
A component of the eukaryotic cytoskeleton. Intermediate filaments form a dense network extending from the nucleus to the plasma membrane.

TERATOCARCINOMA
A malignant germ-cell tumour arising from the ovary or testis that is composed of embryonal carcinoma cells.

INTEGRINS
A family of more than 20 heterodimeric cell-surface extracellular matrix (ECM) receptors. They connect the structure of the ECM with the cytoskeleton and can transmit signalling information bidirectionally.

HEPATIC STELLATE CELLS
The principal fibrogenic cell type of the liver. They are located in a perivascular orientation and contain long cytoplasmic processes that interact with neighbouring cells.

HEMIDESMOSOME
An adhesion complex located at the interface of epithelial cells with the basement membranes. Responsible for linking keratin intermediate filaments to components of the extracellular matrix.

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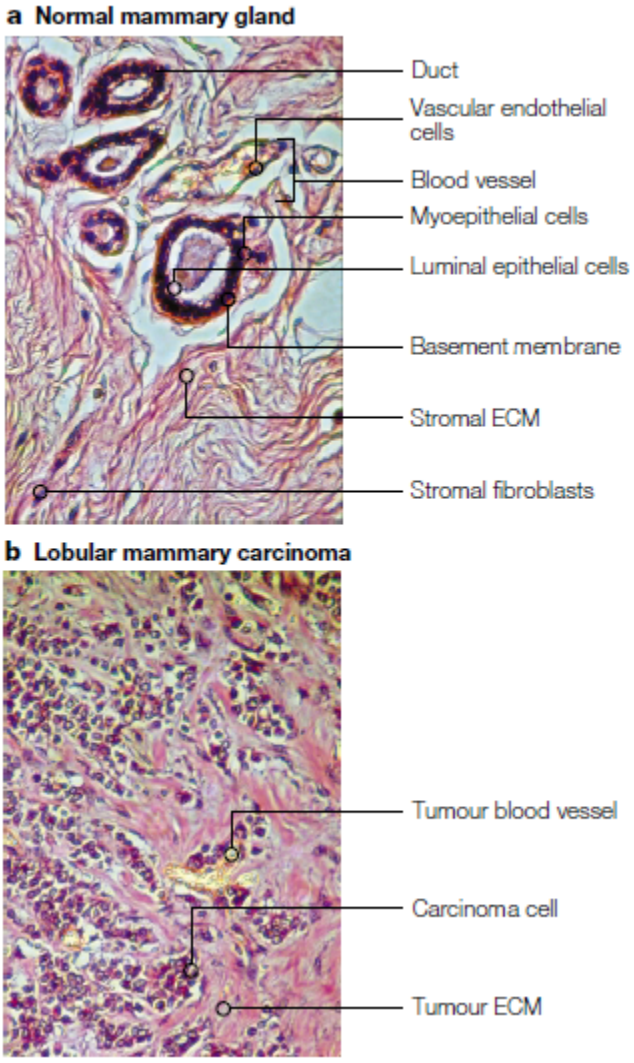
Figures, Boxes, and Tables

FIGURE 1



Normal versus malignant breast tumours.
a | The normal mammary gland shows a highly structured and segregated architecture. Ducts are formed by a double layer of cells: luminal epithelial cells surrounded by a layer of myoepithelial cells, enclosed by the basement membrane. Stromal fibroblasts secrete a collagenous extracellular matrix (ECM), and blood vessels are centrally located and well defined. **b** | Lobular breast carcinoma is less organized. Tumour angiogenesis produces poorly defined blood vessels, and carcinoma cells intermingle with all the stromal elements.

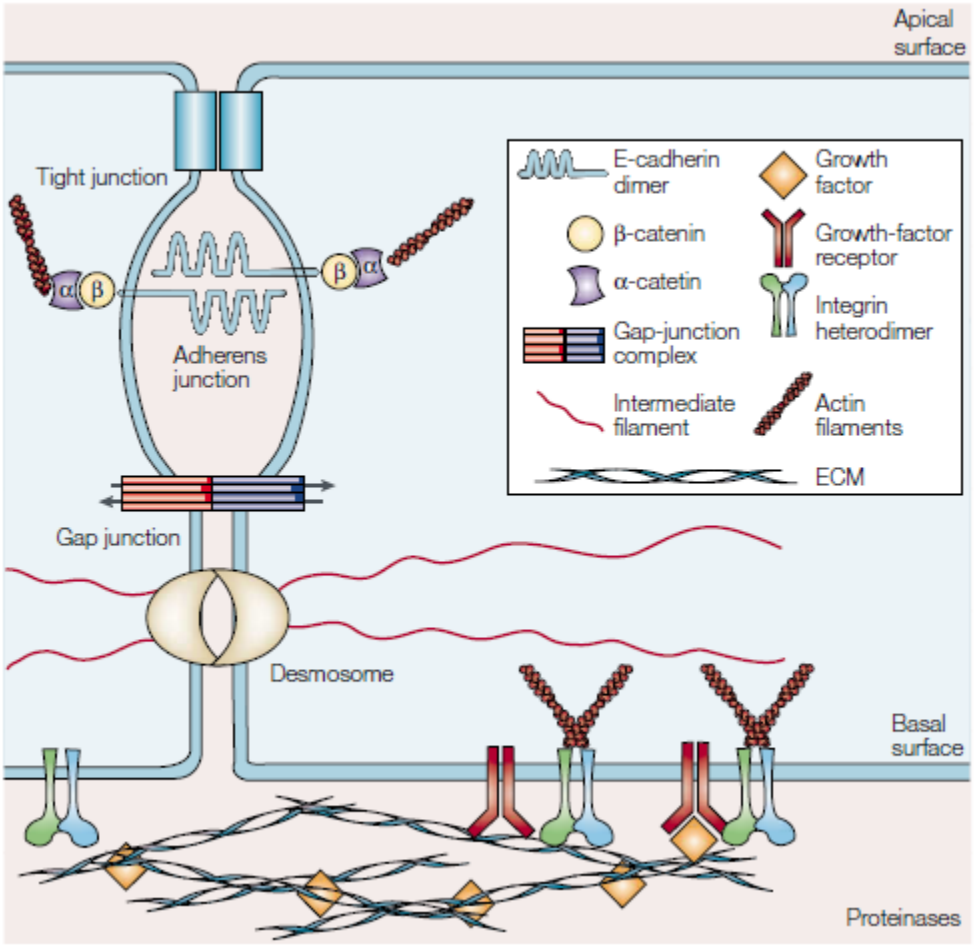
BOX 1

Box 1 | Epithelial cell polarity and tumorigenesis in *Drosophila*

Loss of apicobasal polarity in carcinomas has often been viewed as a secondary consequence of oncogenic transformation[134], but recent investigations of *Drosophila* mutants have shown that loss of polarity determinants can directly lead to increased proliferation and development of tumours. In a genetic screen for mutations that cause aberrant epithelial structures, Bilder, Li and Perrimon[135] identified *scribble* (*Scrib*) as a gene that is required for proper localization of apical proteins and the components of the adherens junctions. Absence of Scrib is associated with the loss of epithelial polarity and neoplastic transformation, a phenotype previously observed in two other *Drosophila* mutants: discs large (Dlg) and lethal giant larvae (Lgl); subsequent investigations showed that all three proteins act in the same pathway[136]. Although many details are not yet resolved, Scrib and Dlg seem to colocalize at the septate junction, a structure similar to the mammalian tight junction, where they direct the polarized sorting of a specific population of Lgl-containing vesicles. It is loss of this directional-sorting mechanism that suppresses normal growth-control mechanisms.

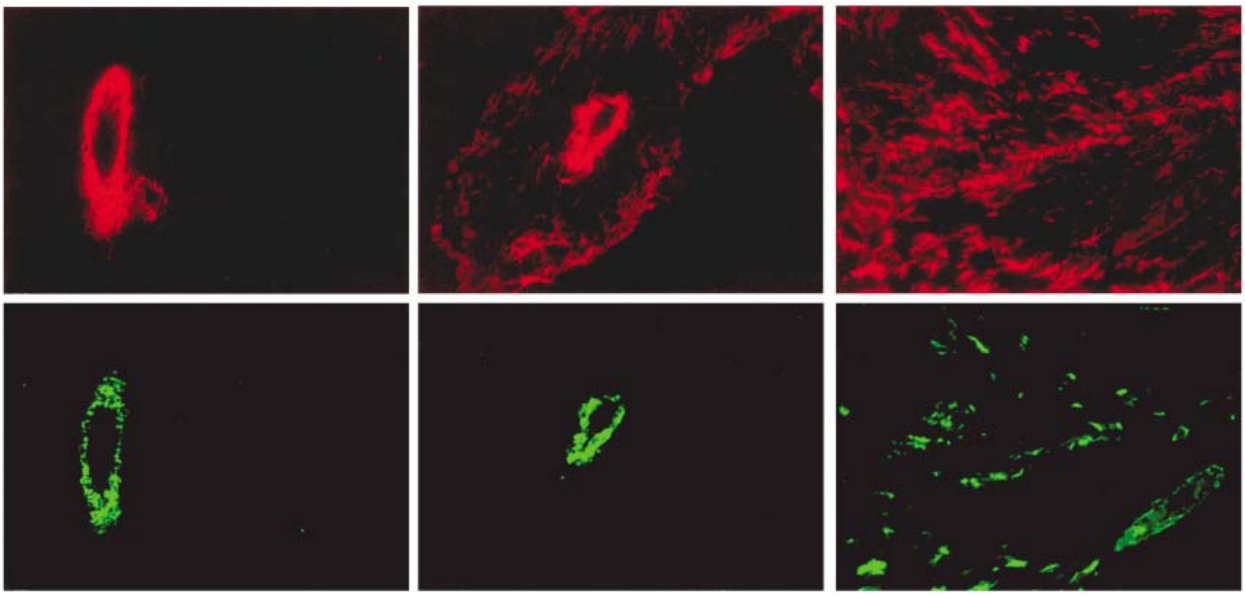
Two mechanisms have been proposed to account for the increased proliferation. First, it is possible that misdirection of cell-surface signal receptors could lead to inappropriate receptor synergy; in this regard, it is noteworthy that ERBIN, a mammalian protein with homology to Scrib, has been shown to interact with the epidermal growth factor receptor (EGFR) family member ERBB2 (HER2)[137]. Alternatively, mislocalization of the components of the adherens junctions might release contact inhibition, leading to hyperproliferation. At present, these mechanisms cannot be distinguished, and it is possible that both contribute to neoplastic transformation. Further investigations of molecules that maintain the tissue-specific functions of polarized epithelia will doubtless reveal more about their complex relationship with tumorigenesis.

FIGURE 2



Mechanisms of cell–cell and cell–ECM interactions. Integrin and non-integrin cell-surface receptors form attachments with the actin filaments in the cytoskeleton, and are able to sense elements of the extracellular matrix (ECM) to promote growth-factor activation. Tight junctions act as a barrier to the diffusion of solutes through the intercellular space and act as a boundary between the apical and basolateral plasma-membrane domains. Adherens junctions, which consist of extracellular E-cadherin dimers connected to cytoplasmic α- and β-catenin molecules, are anchored to actin filaments. Gap junctions provide a communication mechanism by allowing solutes and small signalling molecules to pass between adjacent cells. Desmosomes serve as anchoring points for INTERMEDIATE FILAMENTS and also provide signalling information.

FIGURE 3



Differences in stroma between tumours. Interstitial stromal cells of normal and breast tumour tissues differ in levels of smooth muscle differentiation. **a** | Normal interstital stroma(s) does not express smooth muscle actin (red) or **b** | smooth muscle myosin (green), indicating that smooth muscle differentiation has not taken place, although these cells do produce blood vessels (bv). **c–f** | Tumour tissues, by contrast, express high levels of smooth muscle actin (**c**,**e**). These images also show, however, that not all tumour stroma are similar. The tumour stroma shown in (**c**) and (**d**) expresses smooth muscle actin (**c**) but not smooth muscle myosin (**d**). In the tumour shown in **e** and **f**, the stromal cells express high levels of both actin and smooth muscle myosin. Adapted from REF. 142.

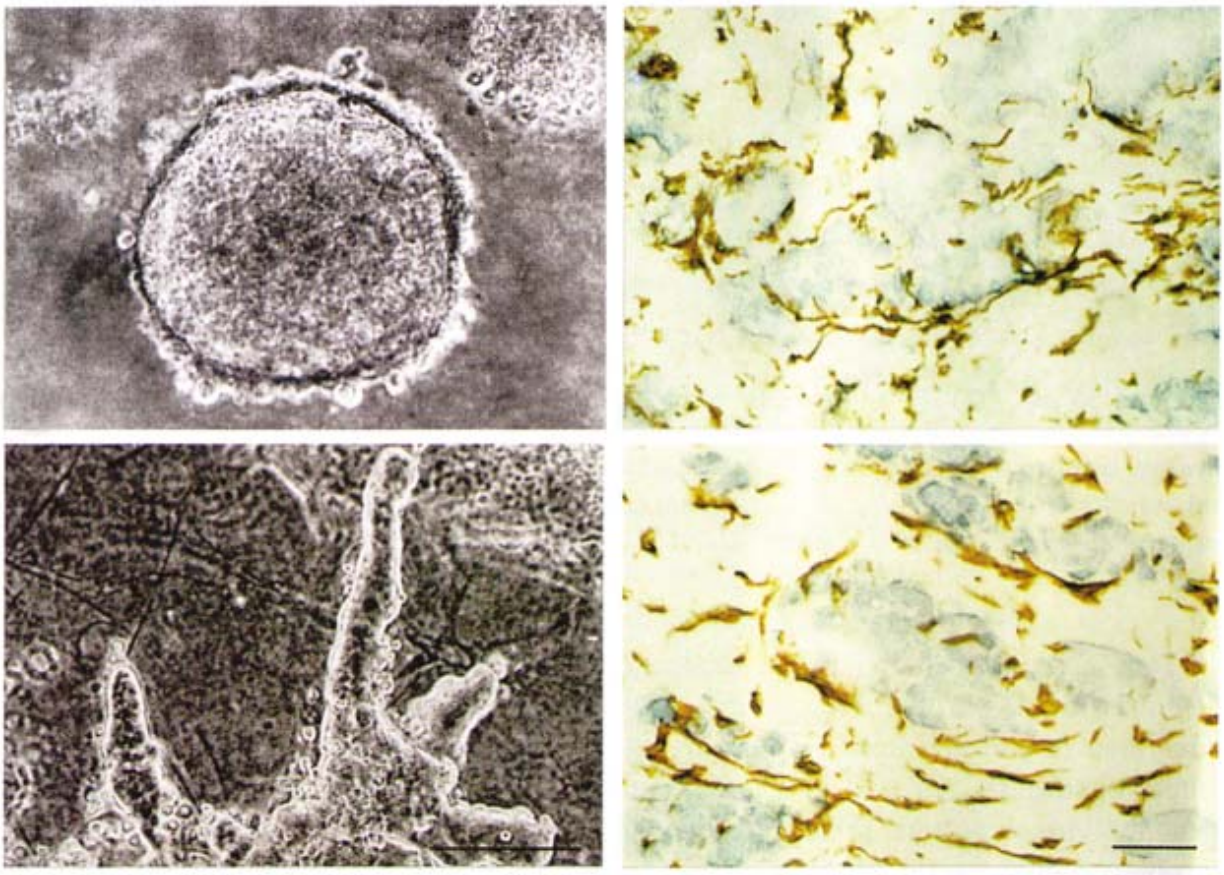
TABLE 1

Table 1 | **Myoepithelial proteins that suppress luminal tumours**

Protein	Function	Reference
α-smooth muscle actin	Cell structure	143
Cytokeratin 5	Cell structure	144
α6-integrin	ECM receptor	145
Caveolin-1	Cell-surface molecule	146
Connexin 43	Gap-junction component	147
Maspin	Protease inhibitor	148
TIMP-1	Protease inhibitor	29
Relaxin	Hormone	149
Activin	Hormone	150

These myoepithelial-specific proteins (which are sometimes expressed in cultured epithelial cells) inhibit epithelial tumour formation, showing that molecules made by myoepithelial cells have tumour-suppressive activities.

FIGURE 4



The tumour microenvironment assay. a | Primary breast carcinoma cells form spherical colonies when cultured in three-dimensional collagen type I. **b** | Co-cultivation with stromal cells, however, causes the tumour cells to spread and become invasive. The degree of tumour growth increases with the density of the stromal cells. Staining of the coculture assay (**c**) and of tumour (**d**) with anti-vimentin antibody reveals the structural similarities of stromal cells in the presence or absence of cancer cells. (Reproduced with permission from REF. 142 © (1995) American Society for Clinical Investigation.)

BOX 2

Box 2 | **Comparison between wound healing and tumour development**

Wound healing and tumour development are dynamic, progressive processes that involve the interaction of several tissue types[138], and comparison of the two reveals many mechanistic similarities. **a** | Immediate reaction to wounding. Tissue injury leads to activation of platelets that form a haemostatic plug and also release vasoactive mediators to increase vascular permeability and to enable the influx of serum fibrinogen to generate the fibrin clot. Platelets produce chemotactic factors, including transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF). These factors initiate the formation of granulation tissue by activating fibroblasts to produce matrix metalloproteinases (MMPs) and a number of growth factors, such as fibroblast growth factor-2 (FGF-2). These factors degrade dermal extracellular matrix, stimulate infiltration of macrophages and promote the development of new blood vessels. These interactions are potentiated by reciprocal signalling between the epidermis and dermal fibroblasts through growth factors, MMPs, and members of the TGF-β family. **b** | Reformation of the epithelial sheet. The complex reaction to wounding reduces epithelial adhesiveness and increases epithelial-cell mobility to re-form an intact sheet of tissue over the wound. Production of MMPs and proteolytic enzymes such as uroplasminogen activator (uPA) and tissue plasminogen activator (tPA) facilitates this re-epithelialization. Blood vessels can then enter the fibrin clot as epidermal cells resurface the wound. The lateral migration of the epidermal cells is followed by a reversion to the normal, non-motile phenotype, including regeneration of a basement membrane and resynthesis of HEMIDESMOSOMES. Following re-epithelialization, a new basement membrane is produced and many of the fibroblasts take on a myofibroblast phenotype to facilitate wound contraction. **c** | Reciprocal activation mechanisms in early tumours. Building on a rich, but inconclusive, literature spanning nearly a century (reviewed in REF. 35), Dvorak proposed that tumours activate some of the normal wound-healing responses[139]. Although developing tumours do not disrupt the vascular tissue in the same way as in wounding, many of the processes occur in parallel. Tumour cells (blue) produce many of the same growth factors that activate the adjacent stromal tissues in wounding or fibrosis[37,140,141]. Activated fibroblasts and infiltrating immune cells (macrophage) secrete MMPs and cytokines such as TGF-β, FGF-2, and PDGF. These factors potentiate tumour growth, stimulate angiogenesis, and induce fibroblasts to undergo differentiation into myofibroblasts and into smooth muscle. **d** | Expression of proteases at the invasive front. Tumour cells, myofibroblasts and activated macrophages increase production of MMPs and uPA at the invasive front to stimulate angiogenesis and proliferation. Production of TGF-β also promotes tumour growth. uPAR, uroplasminogen receptor.